



Scientists discuss historic research

A roundtable discussion at The Rockefeller University recently brought together six pioneers active in the field of genetic research between the publication of the Avery paper and the discovery of the structure of DNA.

Participants in the event, introduced by Professor Norton Zinder and chaired by Visiting Professor Robert Olby, were: Erwin Chargaff, Seymour Cohen, Alfred Hershey, Rollin Hotchkiss, Maclyn McCarty and Joshua Lederberg. Susan Blum of News&Notes presents some of its highlights below. Dr. Olby's questions are in *italic* followed by the participants' responses.

Dr. Cohen, your contact with the nucleic acids antedated the publication of the Avery lab paper in 1944. What were the problems you tackled in those years?

In 1941-42, in the Rockefeller lab of Wendell Stanley, I was studying nucleic acid to find out how it was linked to protein. Before we were finished, I knew that we had a very large molecule indeed, much larger than a tetranucleotide. [The then-popular "tetranucleotide hypothesis" held that nucleic acids were "stupid" molecules with no variation and, thus, no ability to convey information.]

After leaving Rockefeller, I worked for a year in the Columbia lab of Dr. Chargaff. There, as a result of studies with rickettsia, we were able to determine that half of the deoxyribose in DNA was purine and half pyrimidine...

In 1946, Delbrück and Hershey showed that one could make crosses

between bacteriophages. I thought that if the Avery paper (which had greatly impressed me) was correct, I ought to be able to isolate the DNA from one of these bacteriophages, mix it with an intact phage, and, after infecting bacteria with this mixture, get out a genetic cross. Attempts to cross these materials were negative, so I dropped this set of experiments and went on to my true love, that of the biochemical nature of virus multiplication.

It is a remarkable thing that no one undertook to determine whether the RNA of tobacco mosaic virus on its own was infectious [and thus transferred genetic information] until much later. We didn't plumb the full breadth of the Avery results until about 1963.

Dr. Chargaff, looking at the list of your papers one can see that you made a very striking change in your agenda to concentrate upon the nucleic acids. Could you tell us about that?

Avery, MacLeod and McCarty wrote an extremely cautious paper, but I was immediately highly impressed with the idea that it was proof of the specificity of nucleic acids [that is, that DNAs are not all alike]. I decided that the first thing to do was to work out quantitative methods in order to characterize DNA. We proceeded to isolate DNAs from different animal, plant and bacterial sources, and worked out methods for the characterization of all components. [These studies showed that while the base

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composition of DNA varied among species, in the DNA of all species examined the amount of thymine equaled that of adenine, and the amount of cytosine that of guanine. These base ratios were crucial for the discovery of DNA's double-helical structure.]...

To comment on more recent history, I regret that in the course of the creation of molecular biology—which should be the union between biochemistry, biology and genetics—biochemistry has been pushed to the wall more and more. The tremendous number of references to the biological and genetic roles of nucleic acid are not matched by an increasing knowledge of the structure of DNA. For instance, the trace components that are an important part of plant DNA have been completely overlooked. If the compounds are there, there is a reason, and we ought to find it.

Dr. McCarty, when you first met Avery you were given a choice of topics, but you had already made up your mind: the story of bacterial transformation had captured your imagination. Would you comment on this?

Actually, I wasn't given choices of what to do, because Avery didn't do that. Instead, he gave very well organized talks about the lab's work to the young people coming into the lab, and also, of course, he had you read the background material.

Avery didn't get back from his holidays in Maine until about the middle of September, and I'd been in the lab doing some reading since the beginning of the month. In this way, I was quickly brought up to date on the lab's work on pneumococcal transformation, having heard of the phenomenon some years earlier in medical school.

One day, Avery was titrating some of the materials that he and MacLeod had made the spring before, and he asked me if I wanted to join him in the experiment. I was

glad to do it, and I was hooked immediately.

On another matter, ever since we had felt fairly confident that DNA was involved [in transformation], we set about trying to find purified DNase [an enzyme that digests DNA], but we didn't have it by the time we published the '44 paper. The references in the '44 paper to DNase were to a number of sources of crude enzymes—including tissue extracts, pneumococcus lysates and animal sera—which all had something that would destroy the transforming activity of our preparation. So what appeared in that paper was a demonstration that all of those substances, which were active in destroying the transforming substance, were also able to attack DNA. There was a correlation, but that's all we had.

By applying the techniques that had been developed in the Princeton laboratories of The Rockefeller Institute by Kunitz and Northrup, we finally got DNase, and had something to report in 1945. As I was writing that paper, I found a reference to a paper on purified DNase published in a German journal in 1941, and managed to get a copy of it from the Alien Property Custodian of the U.S. Government. If it had been available from the start, we would have had the enzyme to work with before the '44 paper was published. These events weren't a major effect of the war, but they certainly influenced the Avery story.

Dr. Hotchkiss, you have urged this university to recognize with pride that it gave uninterrupted support to the next steps of integration of the Avery discovery into the fabric of classical genetics. Would you enlarge on that?

In the 10 years following the '44 paper, there were two broad questions preoccupying biochemists and geneticists. The first question was: was it DNA, and only DNA, that changed bacteria? The second was: what do those changes in bacteria

have to do with genetics—in particular, the genetics of traits other than those of the cell surface, and the genetics of microorganisms other than bacteria?

Most of the approach to these broad questions was still centered at Rockefeller during the decade preceding Watson and Crick. By 1954, our research at Rockefeller had shown that pneumococcal DNA could be freed of protein and yet remain active. DNA was also shown to behave as a classical array of bacterial genes, including those for traits other than surface antigens.

Dr. Hershey, what comments would you like to make about the work of Avery and his colleagues?

Two things have struck me about the work of Avery, MacLeod and McCarty. First of all, it was wonderful, and still is wonderful. But second, it had so little influence. Why? As long as you're thinking [as a geneticist] about inheritance, who gives a damn what the substance is? It's irrelevant. And once you know that the genetic material is DNA, there's only one inference, that you should study DNA. Dr. Chargaff did, but few others did—until, of course, Watson and Crick.

Another reason for Avery and his colleagues' work not attracting as much attention as it might have is that they were just too modest; they refused to advertise.

There is a third factor, too, not really an explanation, but a curiosity. The pneumococcus was an extremely awkward system to work with. A close analogy is the tobacco mosaic virus, which was the first vehicle for demonstrating the role of nucleic acid in viral infection. These were the last systems you would have chosen if you had been looking for material to study from this point of view.

Dr. Lederberg, what was the state of bacterial genetics at the time you moved into this field? And what was the influence of the Avery lab paper

on your work?

My immediate reaction to the paper, as I wrote in a letter after reading it, was that it was "terrific and unlimited in its implications."

But what to make of it? I was less preoccupied with the chemical identity of the transforming principle than with its biological meaning. I was quite confident that the chemistry would be resolved by a clearly defined path of analytical testing. In retrospect, however, it is hard to recall how vague were our concepts of bacterial cells and bacterial genetics. There were many other competing hypotheses to account for transformation.

I felt that the simplest answer to these dilemmas of interpretation would be to transform *Neurospora* [a type of fungus] in the same paradigm as the pneumococcal studies. So I spent the spring of 1944 trying to transform *Neurospora*, in the lab of Francis Ryan at Columbia University [but this line of experimentation did not lead to transformation of *Neurospora* with DNA].

I concluded that we should turn the problem on its head, and instead look for a system of genetic crossing in bacteria, hitherto unknown, to provide a robust, theoretical framework for the transformation studies. The experimental design [worked out in 1946, while Lederberg was a Ph.D. student in the Yale lab of Edward Tatum] was one that would become quite routine in future years, and amazingly enough it worked—and rather promptly! [Lederberg had discovered a "sexual breeding" system whereby two bacteria conjugate and form a connecting bridge through which one passes a chromosomal strand to the other. Subsequent research with Norton Zinder would show that bacterial genetic material is exchanged not only by conjugation, when the entire complement of chromosomes is transferred from one bacterial cell to another, but also by transduction, when only fragments are transferred].